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(54) MONOCLONAL ANTIBODY TO NOVEL ANTIGEN ASSOCIATED WITH HUMAN TUMORS

MONOKLONALER ANTIKÖRPER GEGEN EIN NEUES HUMANES TUMOR ASSOZIIERTES ANTIGEN

ANTICORPS MONOCLONAL CONTRE UN ANTIGENE NOUVEAU ASSOCIE A DES TUMEURS HUMAINES

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Description

Field of the Invention

The present invention relates to a novel monoclonal antibody and a novel antigen, and to methods for production and use of such novel monoclonal antibody reactive with human carcinoma cells. More specifically, the monoclonal antibody of this invention is reactive with the novel cell surface antigen, which is associated with a variety of human tumors including carcinomas of the breast, colon, ovary and lung, as well as melanomas and sarcomas.

The monoclonal antibody of this invention is suitable for both in vivo and in vitro clinical diagnostic purposes, such as the detection of malignant carcinomas. Additionally the antibody of the present invention is suited for therapeutic uses, for example to react with tumor cells, and in conjugates as a target-selective carrier of various agents which have anti-tumor effects including, but not limited to: chemotherapeutic drugs, toxins, immunological response modifiers, and radioisotopes. The antigen of the invention is also useful for therapeutic and diagnostic purposes.

Background of the Invention

Carcinomas cause millions of deaths annually. For example, lung carcinomas are responsible for the majority of deaths from cancer among men and are overtaking breast carcinomas as the most frequent cause of cancer death among women. Most cases of carcinomas are incurable by chemotherapy and radiation therapy unless radically removed in the early stages of the disease. There is thus a great need for methods of diagnosis and therapy of carcinomas of the breast, colon, ovary and lung, as well as for other malignant neoplasms such as melanomas and sarcomas.

Monoclonal antibodies reactive with carcinoma-associated antigens are known (see, e.g., Papsidero, Semin. Surg. Oncol., 1 (4):171-81 (1985); Schlom et al., Important Adv. Oncol., 170-92 (1985); Allum et al., Surg. Ann., 18:41-64 (1986); Houghton et al., Semin. Oncol. 13 (2):165-79 (1986); Monoclonal Antibodies in Cancer: Advances for Diagnosis and Treatment, Roth (ed.), Futura Publishing, Mt. Kisco, New York (1986); and Cancer Diagnosis In Vitro Using Monoclonal Antibodies, Kupchik (ed.) Marcel Dekker, Inc., New York, (1988)).

Most of the known monoclonal antibodies are reactive with several types of human carcinomas, while a few antibodies react with carcinomas derived from specific organs of the body, e.g., lung, breast, ovary, colon, stomach or pancreas. The target antigens are commonly glycoproteins or glycolipids (see, e.g., Hellstrom et al., Cancer Research 46:3917-23 (1986); and Fink et al., Prog. Clin. Pathol., 9:121-33 (1984)). For example, monoclonal antibodies reactive with glycoprotein antigens on specific types of carcinomas include those described in United States Patent 4,737,579 (monoclonal antibodies to non-small cell lung carcinomas), United States Patent 4,753,894 (monoclonal antibodies to human breast cancer), United States Patent 4,579,827 (monoclonal antibodies to human gastrointestinal cancer), and United States Patent 4,713,352 (monoclonal antibodies to human renal carcinoma). Some monoclonal antibodies react with high molecular weight antigens which appear to be mucins. For example, monoclonal antibody B72.3 appears to recognize a tumor-associated oncofetal glycoprotein antigen of greater than 1,000 kd molecular weight that is selectively expressed on a number of different carcinomas. Thus, B72.3 has been shown to react with 84% of breast carcinomas, 94% of colon carcinomas, 100% of ovarian carcinomas and 96% of non-small-cell lung carcinomas (see Johnston, Acta Cytol., 1 (5):537-56 (1987) and United States Patent 4,612,282, issued to Schlom et al.). Similarly, monoclonal antibody KC-4 recognizes an approximately 400-500 kd protein antigen expressed on a number of carcinomas, such as colon, prostate, lung and breast carcinoma (see United States Patent 4,708,930).

Monoclonal antibodies reactive with glycolipid antigens that are believed to be associated with certain tumor cells have also been disclosed. For example, Young et al., J. Exp. Med., 150:1008-19 (1979) disclose the production of two monoclonal antibodies specific for asialo GM_2, a cell surface glycosphingolipid antigen that was established as a marker for BALB/c 3T3 cells transformed by Kirsten murine sarcoma virus. See, also, Kniep et al., J. Immunol., 131 (3):1591-94 (1983) and United States Patent 4,507,391 (monoclonal antibody to human melanoma).

In addition, monoclonal antibodies reactive with glycolipid antigens found on specific types of carcinoma cells include those described by Rosen et al., Cancer Research, 44:2052-61 (1984) (monoclonal antibodies to human small cell lung cancer); Varki et al., Cancer Research, 44:681-87 (1984) (monoclonal antibodies to human adenocarcinomas of the lung, stomach and colon and melanoma), and United States Patent 4,579,827 (monoclonal antibodies to human colon adenocarcinoma). See, also, Hellstrom et al., Proc. Nat'l. Acad. Sci. USA, 83:7059-63 (1986) which describes the L6 monoclonal antibody that recognizes a carbohydrate antigen expressed on the surface of human non-small cell lung carcinomas, breast carcinomas and colon carcinomas.

Additional monoclonal antibodies exhibiting a reactivity to antigens found on a variety of tumor cells are greatly needed. This is because of the antigenic heterogeneity of most tumors which often necessitates, in diagnosis or therapy, the use of a combination of different monoclonal antibodies directed to the same tumor mass. Furthermore, monoclonal antibodies that display a high degree of reactivity with a wide range of tumors, while showing the absence of or only a very weak reactivity with normal tissues, are not common. Such antibodies would clearly be advantageous.

It is thus apparent that a monoclonal antibody reactive with an antigen expressed at high levels by a variety of tumors may become useful towards an earlier diagnosis of cancers, a better definition of the spread of the cancer, the immunological monitoring of cancer patients, as well as for development of improved methods for therapy of cancers. It is also apparent that monoclonal antibodies to novel cell surface molecules can be used for further definition of such molecules which may be of value for preparing immunogens in the form of cancer vaccines, and which may also have important cellular functions, for example, as receptors of hormones or growth factors or as molecules otherwise involved in intra- and intercellular communication. The antigens may even have enzymatic or growth factor activity by themselves.

Summary of the Invention

The present invention provides such a monoclonal antibody, L45, which is specific for a determinant site on a cell surface glycoprotein antigen, the L45 antigen, associated with a variety of human tumor cells, including lung, breast, ovary and colon carcinoma and melanoma and sarcoma cells. Thus, the antibody of the invention can be useful for the diagnosis and therapy of tumors expressing the L45 antigen identified by antibody L45. The L45 antibody of the invention is of the class IgG, and IgG2a subclass and shows no significant reactivity with normal human cells.

The antibody of the invention may be used in in vitro diagnostic methods for determining the presence of a malignant condition in human lung tissue and other human tissues. The methods involve examining the tissue for the presence of an antigen having the characteristics of the 100,000 dalton L45 antigen glycoprotein reactive with antibody L45. For example, the tissue can be contacted with the L45 monoclonal antibody of the invention which defines a determinant site on a cell-associated antigen having the characteristics of the L45 antigen, a functional equivalent or a fragment of this antibody and any interactions of said antibody and antigenic determinants are detected. One such method involves the determination of the presence of carcinoma cells in a specimen suspected of containing such cells. The specimen is contacted with the monoclonal antibody, which is capable of distinguishing such cells from other cell types which may be present in the specimen. The contact is carried out under conditions for binding of the antibody to such cells. After contact, the presence or absence of binding of the antibody to the cells in the specimen is determined. This binding is related to the presence or absence of carcinoma cells in the specimen. Generally, the specimen is contacted with a labeled specific binding partner of the monoclonal antibody. This label is capable of producing a detectable signal. Alternatively, the monoclonal antibody itself may be labeled.

Another diagnostic method involves the in vivo localization of a tumor by administering to a patient a purified antibody or antibody fragment of the present invention labeled with an agent which gives a detectable signal. The localization is then detected using external scintography, emission tomography or radionuclear scanning. This method can also provide better ways to stage cancer patients with respect to the extent of disease and to monitor changes in response to therapy.

The invention also has therapeutic applications, since the L45 antibody and similar antibodies can react with the L45 antigen that is expressed in high concentrations at the tumor cell surface. The monoclonal antibody of the invention may be used to prepare a composition for treating tumors. The composition comprises a therapeutically effective amount of the antibody in association with a pharmaceutically acceptable parenteral vehicle. The antibody of the invention can also be used in immunoconjugates as a carrier of various agents which have an antitumor effect, including, but not restricted to, chemotherapeutic drugs, toxins, immunological response modifiers, and radioisotopes.

The invention also comprises the novel L45 antigen characterized by a molecular weight of about 100,000 daltons and having an amino terminal amino acid sequence: W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F, in which X represents an unidentified amino acid, and equivalents, identified by antibody L45 and the class of antibodies that bind to this antigen.

The invention includes methods for using the purified or cloned L45 antigen as a vaccine to immunize against certain tumors.

Detailed Description of Invention

In order that the invention herein described may be more fully understood, the following detailed description is set forth.

The present invention concerns a novel monoclonal antibody, designated L45, which is specifically reactive with an antigen (L45 antigen) localized on human tumor cells including carcinomas of the lung, colon, breast, ovary, and melanoma and sarcoma cells, methods for producing the L45 monoclonal antibody and diagnostic and therapeutic methods employing the antibody. The L45 antibody reacts with a range of tumors while showing essentially no reactivity with normal human tissues or other types of tumors such as lymphomas.

The invention further concerns a novel cell surface glycoprotein antigen, designated L45 antigen, associated with human tumors of the lung, breast, colon, ovary, and melanomas and sarcomas and methods for using the L45 antigen.

The monoclonal antibody of the invention can be prepared by hybridoma fusion techniques or by techniques that utilize EBV-immortalization technologies.

Hybridoma fusion techniques were first introduced by Kohler and Milstein (see, Kohler and Milstein, Nature, 256:495-97 (1975); Brown et al., J. Immunol., 127 (2):539-46 (1981); Brown et al., J. Biol. Chem., 255:4980-83 (1980); Yeh et al., Proc. Nat'l. Acad. Sci. (USA), 76 (6):2927-31 (1976); and Yeh et al., Int. J. Cancer, 29:269-75 (1982)).

These techniques involve the injection of an immunogen (e.g., purified antigen or cells or cellular extracts carrying the antigen) into an animal (e.g., a mouse) so as to elicit a desired immune response (i.e., production of antibodies) in that animal. For example, human lung carcinoma cells from pleural effusions, cultured cells from explanted human non-small cell lung carcinomas (NSCLC), or cells from a normal fetal lung or lysates from such cells may be used as the immunogen. In the illustrative example herein, explanted cells from a NSCLC (human lung adenocarcinoma), line CH3, were used as the immunogen. The cells are injected, for example, into a mouse and, after a sufficient time, the mouse is sacrificed and somatic antibody-producing lymphocytes are obtained. Antibody-producing cells may be derived from the lymph nodes, spleens and peripheral blood of primed animals. Spleen cells are preferred. Mouse lymphocytes give a higher percentage of stable fusions with the mouse myelomas described below. The use of rat, rabbit and frog somatic cells is also possible. The spleen cell chromosomes encoding desired immunoglobulins are immortalized by fusing the spleen cells with myeloma cells, generally in the presence of a fusing agent such as polyethylene glycol (PEG). Any of a number of myeloma cell lines may be used as a fusion partner according to standard techniques; for example, the P3-NS1/1-Ag4-1, P3-x63-Ag8.653 or Sp2/O-Ag14 myeloma lines. These myeloma lines are available from the American Type Culture Collection (ATCC), Rockville, Maryland.

The resulting cells, which include the desired hybridomas, are then grown in a selective medium, such as HAT medium, in which unfused parental myeloma or lymphocyte cells eventually die. Only the hybridoma cells survive and can be grown under limiting dilution conditions to obtain isolated clones. The supernatants of the hybridomas are screened for the presence of antibody of the desired specificity, e.g., by immunoassay techniques using the antigen that has been used for immunization. Positive clones can then be subcloned under limiting dilution conditions and the monoclonal antibody produced can be isolated. Various conventional methods exist for isolation and purification of the monoclonal antibodies so as to free them from other proteins and other contaminants. Commonly used methods for purifying monoclonal antibodies include ammonium sulfate precipitation, ion exchange chromatography, and affinity chromatography (see, e.g., Zola et al., in Monoclonal Hybridoma Antibodies: Techniques And Applications, Hurell (ed.) pp. 51-52 (CRC Press 1982)). Hybridomas produced according to these methods can be propagated in vitro or in vivo (in ascites fluid) using techniques known in the art (see, generally, Fink et al., supra, at page 123, Fig. 6-1).

Generally, the individual cell line may be propagated in vitro, for example in laboratory culture vessels, and the culture medium containing high concentrations of a single specific monoclonal antibody can be harvested by decantation, filtration or centrifugation. Alternatively, the yield of monoclonal antibody can be enhanced by injecting a sample of the hybridoma into a histocompatible animal of the type used to provide the somatic and myeloma cells for the original fusion. Tumors secreting the specific monoclonal antibody produced by the fused cell hybrid develop in the injected animal. The body fluids of the animal, such as ascites fluid or serum, provide monoclonal antibodies in high concentrations. As discussed by Cole et al., supra, when human hybridomas or EBV-hybridomas are used, it is necessary to avoid rejection of the xenograft injected into animals such as mice. Immunodeficient or nude mice may be used or the hybridoma may be passaged first into irradiated nude mice as a solid subcutaneous tumor, cultured in vitro and then injected intraperitoneally into pristane primed, irradiated nude mice which develop ascites tumors secreting large amounts of specific human monoclonal antibodies (see Cole et al., supra).

For certain therapeutic applications chimeric (mouse-human) or human monoclonal antibodies may be preferable to murine antibodies, because patients treated with mouse antibodies generate human antimouse antibodies. (Shawler et al., J. Immunol. 135:1530-35 (1985)). Chimeric mouse-human monoclonal antibodies reactive with the L45 antigen can be produced, for example, by techniques recently developed for the production of chimeric antibodies (Oi et al., Biotechnologies 4(3):214-221 (1986); Liu et al., Proc. Nat'l. Acad. Sci. (USA) 84:3439-43 (1987)). Accordingly, genes coding for the constant regions of the murine L45 antibody molecule are substituted with human genes coding for the constant regions of an antibody with appropriate biological activity (such as the ability to activate human complement and mediate ADCC). Novel antibodies of mouse or human origin, can be also made to the L45 antigen having the appropriate biological functions. For example, human monoclonal antibodies may be made by using the antigen, e.g. the L45 antigen of the invention, to sensitize human lymphocytes to the antigen in vitro followed by EBV-transformation or hybridization of the antigen-sensitized lymphocytes with mouse or human lymphocytes, as described by Borrebaeck et al. (Proc. Nat'l. Acad. Sci. (USA)) 85:3995-99 (1988)).

According to a preferred embodiment, the antibody of this invention, designated L45, was produced via hybridoma techniques using a lung adenocarcinoma cell line CH3 as the immunogen as described in the Example, infra. The L45 hybridoma, producing the L45 antibody, has been deposited with the ATCC, Rockville, Maryland, and has there been identified as follows:

L45 Accession No.: HB 9804

The L45 antibody is of the IgG2a subclass. The antibody displays a very strong reactivity with tumor cells of a variety of types, for example, carcinomas of the breast, lung, colon and ovary, as well as with malignant melanomas and sarcomas. The L45 antibody shows no detectable binding to the cell lymphoma cell lines, CEM, MOLT-4, and the B cell lymphoma line P3HR-1.

In addition, the antibody of this invention does not display any immunohistologically detectable binding to normal human tissues such as fibroblasts, endothelial cells or epithelial cells from the major organs, e.g., kidney, spleen, liver, skin, lung, breast, colon, brain, thyroid, heart, lymph nodes or ovary. Nor does the antibody react with peripheral blood leukocytes. Thus, this antibody is superior to most known antitumor antibodies in its specificity for a range of tumor cells and in its high degree of specificity for tumor cells as compared to normal cells (see, e.g., Hellstrom et al., Covalently Modified Antigens And Antibodies In Diagnosis And Therapy, Quash/Rodwell (eds.), pp. 24-28 (Marcel Dekker, Inc., (1989); and Bagshawe, Br. J. Cancer, 48:167-75 (1983)).

It should be understood that the present invention encompasses the L45 antibody described above and any fragments thereof containing the active binding region of the antibody, such as Fab, $F(ab')_2$ and Fv fragments. Such fragments can be produced from the L45 antibody using techniques well established in the art (see, e.g., Rousseaux et al., in Methods Enzymol., 121:663-69 Academic Press, (1986)).

In addition, the present invention encompasses antibodies that are capable of binding to the same antigenic determinant as the L45 antibody and competing with the L45 antibody for binding at that site. These include antibodies having the same antigenic specificity as the L45 antibody but differing in species origin, isotype, binding affinity or biological functions (e.g., cytotoxicity). For example, class, isotype and other variants of the antibody of the invention may be constructed using recombinant class-switching and fusion techniques known in the art (see, e.g., Thammana et al., Eur. J. Immunol., 13:614 (1983); Spira et al., J. Immunol. Meth., 74:307-15 (1984); Neuberger et al., Nature, 312:604-08 (1984); and Oi et al., supra)). Thus, chimeric antibodies or other recombinant antibodies (e.g., antibody fused to a second protein such as a lymphokine) having the same binding specificity as the L45 antibody fall within the scope of this invention. Furthermore, since the L45 antigen to which the antibody of the invention binds is a novel pan-tumor antigen, the antibody of the invention includes antibodies that bind to any antigenic determinant on that L45 antigen, including determinants other than that with which the L45 antibody reacts.

Also included within the scope of the invention are anti-idiotypic antibodies of the L45 antibody of the invention. These anti-idiotypic antibodies can be produced using the L45 antibody as immunogen and are useful for diagnostic purposes in detecting humoral response to tumors and in therapeutic applications, e.g., in a vaccine, to induce an antitumor response in patients (see, e.g., Nepom et al., Cancer And Metastasis Reviews, 6:487-501 (1987); and Lee et al., Proc. Nat'l. Acad. Sci. (USA), 82:6286-90 (1985)).

The L45 antibody can be used to isolate and characterize the L45 antigen to which it binds. Thus, L45 can be used as a probe to identify and characterize the epitope recognized by the antibody and to further define the L45 antigen on the surface of the carcinoma cells (see, e.g., Hakomori, Ann. Rev. Immunol., 2:103-26 (1984); Brown et al., J. Immunol., 127: 539-546 (1981); Brown et al., Nature, 296: 171-173 (1982); and Rose et al.; Proc. Nat'l. Acad. Sci. (USA), 83: 1261-1265 (1986)).

The L45 antigen recognized by the monoclonal antibodies of the present invention comprises a novel cell surface glycoprotein antigen characteristic of tumor cells including carcinomas of the breast, colon, ovary and lung as well as melanomas and sarcomas. L45 antigen has a molecular weight of about 100,000 daltons when subjected to immunoprecipitation on polyacrylamide gel electrophoresis.

The amino terminal amino acid sequence of the novel L45 glycoprotein antigen is as follows:

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1         5         10        15        20        25
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
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in which X represents an amino acid that has not been identified as yet, and the rest of the letters represent the conventional single letter abbreviations for amino acids. A comparison of the 26 residue L45 amino-terminal sequence with those stored in the current protein data base (PIR Release 16, March 1988) reveals no significant sequence homology with any other known sequences.

The monoclonal antibody of the invention is also useful for diagnostic applications, both in vitro and in vivo, for the detection of human tumors carrying the L45 antigen with which the L45 antibody is specifically reactive. In vitro diagnostic methods are well known in the art (see, e.g., Roth, supra, and Kupchik, supra), and include immunohistological detection of tumor cells (e.g., on human tissue, cells or excised tumor specimens) or serologic detection of tumor-associated antigens (e.g., in blood samples or other biological fluids).

Immunohistological techniques involve contacting a biological specimen such as a tumor tissue specimen with the antibody of the invention and then detecting the presence on the specimen of the antibody complexed to its antigen. The formation of such antibody-antigen complexes with the specimen indicates the presence of tumor cells in the tissue. Detection of the antibody on the specimen can be accomplished using techniques known in the art such as the immunoperoxidase staining technique, the avidin-biotin (ABC) technique or immunofluorescence techniques (see, e.g., Ciocca et al., Meth. Enzymol. 121:562-79 (1986); Hellstrom et al., Cancer Research, 46:3917-23 (1986); and Kimball (ed.), Introduction To Immunology (2nd Ed.), pp. 113-117, Macmillan Publ. Co. (1986)). For example, immunoperoxidase staining was used as described in Example III, infra, to demonstrate the reactivity of the L45 antibody with lung, breast, colon, and ovary carcinomas and melanomas and sarcomas, and the lack of reactivity of the antibody with normal human tissue specimens.

Serologic diagnostic techniques involve the detection and quantitation of tumor-associated antigens that have been secreted or "shed" into the serum or other biological fluids of patients thought to be suffering from carcinoma. Such antigens can be detected in the body fluids using techniques known in the art such as radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA) wherein an antibody reactive with the "shed" antigen is used to detect the presence of the antigen in a fluid sample (see, e.g., Uotila et al., J. Immunol. Methods, 42:11 (1981) and Allum et al., supra, at pp. 48-51). These assays, using the L45 antibody disclosed herein, can therefore be used for the detection in biological fluids of the L45 antigen with which the L45 antibody reacts and thus the detection of various carcinomas and melanomas in human patients. Thus, it is apparent from the foregoing that the L45 antibody of the invention can be used in most assays involving antigen-antibody reactions. These assays include, but are not limited to, standard RIA techniques, both liquid and solid phase, as well as ELISA assays procedures, immunofluorescence techniques, and other immunocytochemical assays (see, e.g., Sikora et al. (eds.), Monoclonal Antibodies, pp. 32-52, Blackwell Scientific Publications, (1984)).

The L45 antibody of the invention is also useful for in vivo diagnostic applications for the detection of human tumors. One such approach involves the detection of tumors in vivo by tumor imaging techniques using the antibody labeled with an appropriate imaging reagent that produces detectable signal. Imaging reagents and procedures for labeling antibodies with such reagents are well known (see, e.g., Wensel and Meares, Radio Immunoimaging and Radioimmunotherapy, Esevier, New York (1983); Colcher et al., Meth. Enzymol., 121:802-16 (1986)). The labeled antibody may be detected by a technique such as radionuclear scanning (see, e.g., Bradwell et al. in Monoclonal Antibodies for Cancer Detection and Therapy, Baldwin et al. (eds.), pp. 65-85, Academic Press (1985)).

According to another aspect of the invention a method for localizing human tumors in vivo is provided, comprising:

a) purifying the monoclonal antibody of the present invention;
b) radiolabeling said antibody;
c) administering said antibody to a human patient in a suitable carrier; and
d) localizing the monoclonal antibody by external scintigraphy, emission tomography or radionuclear scanning.

The L45 antibody of the invention has a number of in vivo therapeutic applications. In addition to being used alone to target tumor cells, the antibody can be used in conjunction with an appropriate therapeutic agent to treat human cancer. For example, the antibody can be conjugated or linked to a therapeutic drug or toxin for delivery of the therapeutic agent to the site of the cancer. Techniques for conjugating such therapeutic agents to antibodies are well known (see, e.g., Arnon et al., Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56, Alan R. Liss, Inc., (1985); Hellstrom et al., in Controlled Drug Delivery (2nd ed.), Robinson et al. (eds.), pp. 623-53, Marcel Dekker, Inc., (1987); Thorpe, Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., Immunol. Rev., 62:119-58 (1982)). Since the L45 antibody is not easily internalized when cells are exposed to it in vitro, it may be preferable to target chemotherapeutic drugs to the tumor cells by coupling the antibody with an enzyme, e.g., using recombinant DNA techniques. When such conjugates are localized to the tumor, the enzyme can convert an inactive (nontoxic) prodrug which is administered after the conjugates have bound to the tumor cells, to an active anticancer drug. (See, e.g., Senter et al., Proc. Nat'l. Acad. Sci. (USA), 85:4842-46 (1988)).

Alternatively, the antibody can be coupled to high-energy radiation, e.g., a radioisotope such as ^{131}I, which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order, in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 Academic Press, (1985)). According to yet another embodiment, the L45 can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

Still other therapeutic applications for the L45 antibody of the invention include its use, either in the presence of complement or as part of an antibody-drug or antibody-toxin conjugate, to remove tumor cells from the bone marrow of cancer patients. According to this approach, autologous bone marrow may be purged ex vivo by treatment with the antibody and the marrow infused back into the patient (see, e.g., Ramsay et al., J. Clin. Immunol., 8(2):81-88 (1988)).

Furthermore, chimeric or other recombinant L45 antibodies of the invention, as described earlier, may be used therapeutically. For example, a fusion protein comprising at least the antigen-binding region of the L45 antibody joined

to at least a functionally active portion of a second protein having anti-tumor activity, e.g., a lymphokine or oncostatin, may be used to treat human tumors in vivo. In addition, a chimeric L45 antibody wherein the antigen-binding region of L45 is joined to a human Fc region, e.g., IgG1, may be used to promote antibody-dependent cellular cytotoxicity or complement mediated cytotoxicity. Furthermore, recombinant techniques known in the art can be used to construct bispecific antibodies wherein one of the binding specificities of the antibody is that of L45 (see, e.g., United States Patent 4,474,893).

Finally, anti-idiotypic antibodies of the L45 antibody may be used therapeutically in active tumor immunization and tumor therapy (see, e.g., Hellstrom et al., "Immunological Approaches To Tumor Therapy Monoclonal Antibodies, Tumor Vaccines, And Anti-Idiotypes", in Covalently Modified Antigens And Antibodies In Diagnosis And Therapy, supra, at pp. 35-41).

It is apparent therefore that the present invention encompasses pharmaceutical compositions, combinations and methods for treating human tumors. For example, the invention includes pharmaceutical compositions for use in the treatment of human tumors comprising a pharmaceutically effective amount of a L45 antibody and a pharmaceutically acceptable carrier. The compositions may contain the L45 antibody, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody) or in a recombinant form (e.g., chimeric or bispecific L45). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The antibody compositions of the invention can be administered using conventional modes of administration including, but not limited to, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The antibody compositions of the invention may be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

The antibody compositions also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

The most effective mode of administration and dosage regimen for the compositions of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the compositions should be titrated to the individual patient. Nevertheless, an effective dose of the antibody compositions of this invention may be in the range of from about I to about 5000 mg/m^2.

The novel antigen of the present invention, referred to as antigen L45 may also be used for therapeutic applications. The antigen can be purified from tumors or produced by recombinant DNA technology (Brown et al., copending U.S. Patent Application Serial No. 827,313, Attorney Docket No. 5624-008, filed on February 7, 1986, incorporated by reference herein). The gene coding for the L45 antigen may be cloned by methods which first enrich the mRNA of the L45 antigen. By one such method, polysomes (consisting of mRNA ribosomes and nascent polypeptide chains) can be purified by immunoaffinity chromatography with antibody that recognizes the L45 antigenic determinant on the nascent chain. The mRNA is isolated by immunoprecipitation with, e.g., L45 antibody and the cDNA is cloned in an appropriate expression vector. Alternatively, L45 antibody or antiserum to L45 antigen might be used to screen a cDNA library using an expression vector. The purified or cloned L45 antigen may be administered alone as an immunogen or together with a proper immunological adjuvant.

Purified or cloned L45 antigen may be used in the methods of the invention as a vaccine to immunize against certain tumors. Procedures for preparing such vaccines are known in the art (see, e.g., Estin et al., Proc. Nat'l. Acad. Sci. (USA), 85:1052 (1988)). Briefly, recombinant viruses are constructed for expression of the cloned tumor-associated antigen, for example L45 antigen. Cells infected with the recombinant viruses will express the tumor antigen at the surface of the cells together with the host's incompatibility antigens and immunogenic viral proteins. This favors the induction of cellular immunity which plays a key role in tumor rejection. A suitable virus, for example vaccinia virus derived from a plaque-purified virus of the Wyeth smallpox vaccine (New York City Board of Health strain), is used to construct a recombinant virus containing the coding sequence of the L45 antigen under control of the vaccinia virus "7.5 K" promoter (Hu et al., J. Virol. 62:176-180 (1988). The recombinant virus may then be administered intravenously as a vaccine to protect against cancer.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

EXAMPLE I

Preparation of the L45 Monoclonal Antibody

The L45 monoclonal antibody of the invention was produced using hybridoma fusion techniques described previously by Yeh et al., Proc. Nat'l Acad. Sci. (USA) (1979), supra). Briefly, a three month-old BALB/c mouse was immunized using explanted cultured cells from a human adenocarcinoma of the lung, designated CH3, as the immunogen. The mouse received six (6) intraperitoneal (i.p.) injections and approximately 10^7 cells for each immunization. Three days after the last immunization, the spleen was removed, and the spleen cells were suspended in culture medium. The spleen cells were then fused with transfected NS1 mouse myeloma cells with geniticin (Kohler and Milstein, supra), using polyethylene glycol (PEG), and the cell suspension grown in microtiter wells in selective HAT medium as described by Yeh et al., Proc. Nat's. Acad. Sci. (USA), supra. The mixture was seeded to form low density cultures originating from single fused cells or clones.

The supernatants from these hybridoma cultures were then screened for direct binding activity on the lung cancer cell line, CH3, and against short-term cultures of human fibroblasts using an ELISA assay similar to that described by Douillard et al., Meth. Enzymol., 92:168-74 (1983). According to this assay, the antigen (with which the antibody being screened for is reactive) is immobilized on microtiter plates and then incubated with hybridoma supernatants. If a supernatant contains the desired antibody, the antibody will bind to the immobilized antigen and is detected by addition of an anti-immunoglobulin antibody-enzyme conjugate and a substrate for the enzyme which leads to a measurable change in optical density.

For this example, lung cancer cells or control fibroblast cells or peripheral blood leukocytes (PBLs) were dispensed into a 96-well tissue culture plate (Costar, Cambridge, MA) and incubated overnight in a humid 37°C incubator (5% $C0_2$). The cells were then fixed with 100 µl of freshly prepared 1.0% glutaraldehyde to a final well concentration of 0.5% and incubated for 15 min at room temperature, followed by washing three times with 1 X PBS. The cells were next blocked for 30 min with 5% BSA in PBS and washed again three times with PBS. The supernatants from the hybridoma cultures were then added at 100 µl/well, the wells incubated for 1 hr at room temperature, and the cells washed three times with PBS. Next, goat anti-mouse horseradish peroxidase (Zymed, CA) diluted in 0.1% BSA and PBS was added to a concentration of 100 µl/well. The reaction mixture was incubated for either 1 hr at room temperature or 30 min at 37°C and the cells were then washed three times with PBS. o-phenylenediamine (OPD) was then added at 100 µl/well and the plates incubated in the dark at room temperature for 5-45 min. Antibody binding to the cells was detected by a color change in the wells that occurred within 10-20 min. The reaction was stopped by adding 100 µl/well H_2SO_4 and the absorbance read in a Dynatech (Alexandria, VA) Microelisa autoreader at 492 nm.

The wells still positive on immunizing cell lines and negative on PBLs were tested by immunohistology technologies on immunizing cell line pellets and normal kidney, liver, and spleen tissues.

It should be noted that this assay can be performed using intact cells or purified soluble antigen or cellular extracts as the immobilized antigen. When soluble antigen or cell extracts were used as antigen, the antigen was initially plated at 50 µl/well in PBS and the plates were incubated overnight at room temperature before beginning the assay. When using intact cells as antigen, they may be used fresh or after fixation. In either case, the cells were initially plated at 10^4 cells at 100 µl/well in culture medium and incubated overnight in a 37°C incubator (5% $C0_2$).

Hybridomas which produced antibodies binding to the lung cancer cell line and not to the normal tissues were thus selected, cloned, expanded in vitro, and further tested for antibody specificity. Those hybridomas which produced antibody reactive with human lung cancer were recloned, expanded, and injected into pristane-primed 3-month old BALB/c mice, where they grew as ascites tumors.

Following this procedure, hybridoma cell line L45 was obtained, cloned and injected into mice to develop as an ascites tumor. As disclosed above, the L45 hybridoma has been deposited with the ATCC. Antibody secreted into the ascites was purified on protein A-Sepharose (see, e.g., Ey et al., Immunochemistry, 15:429-436 (1978)) or by gel filtration on Sephacryl S-300. Purified L45 antibody was used for further characterization.

EXAMPLE II

Characterization of The L45 Monoclonal Antibody Isotype Determination

To determine the class of immunoglobulin produced by the L45 hybridoma, the following techniques were utilized:

a) Ouchterlony immunodiffusion.

An aliquot of supernatant of the L45 hybridoma cells was placed into the center well of a 25% agar plate. Monospecific rabbit anti-mouse Ig isotypes antibodies (Southern Biotechnology, Birmingham, AL) were placed in the outer wells and the plate was incubated for 24-48 hr at 37°C. Precipitation lines were then read.

b) ELISA isotyping.

Dynatech Immulon 96-well plates were coated with goat anti-mouse Ig antibodies at 1 µg/ml concentration, 50 µl/well in PBS and left covered overnight at 4°C. The plates were washed with PBS/Tween 20, 0.05% and blocked with medium 100 µl/well for 1 hr at room temperature. After washing the plates, supernatants from the L45 hybridoma were added and incubated at room temperature for 1 hr. After washing with PBS containing bovine serum albumin (BSA) plates were incubated at 37°C for 2 hr with monospecific rabbit anti-mouse Ig isotype antibodies coupled to peroxidase (Zymed). After washing, plates were incubated with 1 mg/ml o-phenylenediamine and 0.03% H_2O_2 in 0.1 M citrate buffer, pH 4.5. Optical density at 630 nm was determined on a Dynatec ELISA plate reader.

Based on these procedures, it was determined that the L45 monoclonal antibody is of the IgG2a isotype.

Binding Characteristics of The L45 Monoclonal Antibody

The subcellular localization of antigen was determined by measuring antibody binding to cells before or after permeabilization with non-ionic detergent. Antibodies binding to the cell surface of intact cultured cells were identified by direct fluorescence using the fluorescence activated cell sorter (FACS) II, as described by Hellstrom et al., Cancer Research 46:3817-3923 (1986). Briefly, for binding analyses using a FACS cell sorter, 1 x 10^6 cultured cells were aliquoted in 15% fetal bovine serum (FBS) in IMDM media (Gibco, Grand Island, NY) to a total volume of 500 µl/tube. The cells were centrifuged for 1.5 min on a Serofuge and the supernatant removed. 100 µl of the L45 monoclonal antibody at 10 µg/ml was added to each tube, the contents of which was then mixed and incubated on ice for 30 min. The reaction mixture was washed three times with 500 µl of 15% FBS/IMDM by centrifugation for 1.5 min on the Serofuge (tubes were blotted after the third wash). Then, 50 µl of optimized FITC-conjugated goat anti-mouse IgG antibody (Tago, Burlingame, CA) diluted 1:25 in 15% FBS/IMDM was added to each tube and the reaction mixture was mixed and incubated for 30 min. The wash step was then repeated and after blotting of the tubes, each pellet was resuspended in 200-500 µl of PBS. Each sample was run on a Coulter Epics C FACS and the mean fluorescence intensity (MFI) was determined. From the MFI, the linear fluorecent equivalent (LFE) was determined. The LFE of each test sample divided by the LFE of a negative control gave a ratio between the brightness of cells stained by specific vs. control antibody (1.0 = no difference in fluoresence, 2.0 = fluoresence twice as bright, etc.). The binding data is shown in Table 1 below.

Table 1

Binding of L45 Antibody to Various Cell Lines	
Cell Lines	L45 Antibody Binding Ratio
2981 Lung carcinoma (ca.)	15.6
CH3 lung ca.	3.3
2707 lung ca.	1.0
HCT116 lung ca.	7.4
3477 breast ca.	13.5
RCA colon ca.	21.0
3347 breast ca.	1.6
C colon ca.	24.3
CEM T lymphocytes	1.0
MOLT 4 T lympocytes	1.3
P34R-1 B lymphoma	1.0
Peripheral blood cells	1.0

As Table 1 demonstrates, the L45 monoclonal antibody reacted with lung, breast and colon carcinoma cell lines, but did not react with T or B lymphoma lines nor with normal peripheral blood leukocytes.

Immunohistology

The PAP technique of L. A. Sternberger as described in Immunochemistry, pp. 104-69, John Wiley & Sons, New York (1979), as modified by Garrigues et al., Int. J. Cancer, 29:511-15 (1982), was used for immunohistological studies on frozen tissue sections. The target tissues for these tests were obtained at surgery and frozen within 4 hr of removal using isopentane precooled in liquid nitrogen. Tissues were then stored in liquid nitrogen or at -70°C until used. Frozen sections were prepared, air-dried, treated with acetone and dried again (see Garrigues et al., supra). Sections to be used for histologic evaluation were stained with hematoxylin. To decrease non-specific backgrounds, sections were pre-incubated with normal human serum diluted 1/5 in PBS (see Garrigues et al., supra). Mouse antibodies, rabbit anti-mouse IgG, and mouse PAP were diluted in a solution of 10% normal human serum and 3% rabbit serum. Rabbit anti-mouse IgG (Sternberger-Meyer Immunochemicals, Inc., Jarettsville, MD) was used at a dilution of 1/50. Mouse peroxidase-antiperoxidase complexes (PAP, Sternberger-Meyer Immunochemicals, Inc.) containing 2 mg/ml of specifically purified PAP were used at a dilution of 1/80.

The staining procedure consisted of treating serial sections with either specific antibody, i.e., L45, or a control antibody for 2.5 hr, incubating the sections for 30 min at room temperature with rabbit anti-mouse IgG diluted 1/50 and then exposing the sections to mouse PAP complexes diluted 1/80 for 30 min at room temperature. After each treatment with antibody, the slides were washed twice in PBS.

The immunohistochemical reaction was developed by adding freshly prepared 0.5% 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO) and 0.01% H_2O_2 in 0.05 M Tris buffer, pH 7.6, for 8 min (see Hellstrom et al., J. Immunol., 127:57-60 (1981)). Further exposure to a 1% OsO_4 solution in distilled water for 20 min intensified the stain. The sections were rinsed with water, dehydrated in alcohol, cleared in xylene, and mounted on slides. Parallel sections were stained with hematoxylin.

The slides were each evaluated under code and coded samples were checked by an independent investigator. Typical slides were photographed by using differential interference contrast optics (Zeiss-Nomarski). The degree of antibody staining was evaluated as 0 (no reactivity), + (a few weakly positive cells), ++ (at least one third of the cells positive), +++ (most cells positive), ++++ (all cells strongly positive). Because differences between + and 0 staining were less clear cut than between + and ++ staining, a staining graded as ++ or greater was considered "positive". Both neoplastic and stroma cells were observed in tumor samples. The staining recorded is that of the tumor cells because the stroma cells were not stained at all or were stained much more weakly than the tumor cells.

Table 2 below presents the immunohistological staining of various tumor and normal tissue specimens using the L45 monoclonal antibody. As the table clearly demonstrates, the L45 antibody reacts with a wide range of human tumor specimens, and shows no reactivity with any of the number of normal human tissues tested.

TABLE 2

Immunoperoxidase Staining of Tumors and Normal Tissue Specimens with L45 Monoclonal Antibody		
Tissue Type		Antibody Binding (Number of Positive Tumors/Total Number of Tumors Tested)
CA. COLON		13/13
CA. LUNG		10/13
CA. BREAST		15/15
CA. OVARIAN		5/6
MELANOMA		8/8
SARCOMA		4/6
Normal Tissues:	SPLEEN	0/3
	KIDNEY	0/4
	LIVER	0/4
	HEART	0/1
	OVARY	0/1
	ADRENAL	0/1
	TESTIS	0/1
	BREAST	0/8
	TONSIL	0/1
	SKIN	0/5
	LUNG	0/5
	COLON	0/6
	BRAIN	0/2
	THYROID	0/3
	LYMPH NODES	0/2

EXAMPLE III

L45 Antigen Recognized By L45 Antibody

Purification

In order to characterize the antigen reactive with the L45 antibody, L45 antigen was isolated from A549 cells (ATCC, Rockville, MD) and purified by immunoaffinity chromatography. L45 antigen was solubilized from A549 cell membranes with 0.5% NP40 in 10 mM Tris-HCl buffer, pH 8.5, containing 0.15 M NaCl and I mM. phenylmethane-sulfonylfluoride and purified by immunoaffinity chromatography. The column was prepared by coupling L45 antibody to carbonyldiimidazole-activated agarose (Reacti-Gel (6X), Pierce, Rockford, IL). Bound L45 antigen was reluted from the affinity support with 0.5 M glycine-HCl buffer, pH 2.3, containing 0.5% NP40 and 0.15 M NaCl. Eluted L45 antigen was further purified by preparative sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 15% acrylamide gel. Following electrophoresis, the gel was stained with Coomassie Brilliant Blue, 0.5% by weight in 10% acetic acid and 30% isopropanol) and destained in a solution of acetic acid (5%, v:v) and methanol (17%, v:v). The stained L45 antigen band (Mr 100,000) was excised with a razor blade and immediately subjected to electroelution with a ECU-040

Electroelutor/Concentrator (C.B.S. Scientific Co., San Diego, CA), as described by Hunkapiller, et al., Methods in Enzymology 91:227-236 (1983)).

Sequence Analysis

Automated Edman degradation was performed with two preparations of L45 antigen: 1) 36 pmol of antigen and 2) 14 pmol of antigen in a pulsed liquid protein sequencer (Model 475A, Applied Biosystems, Inc., Foster City, CA). The phenylthiohydantoin amino acid derivatives were analyzed by reversed-phase high performance liquid chromatography (HPLC) on-line on a Model 120A on-line HPLC unit (Applied Biosystems, Inc.) using a PTH C18 column and a sodium acetate/tetrahydrofuran/acetonitrile gradient for elution.

The amino-terminal amino sequence of L45 antigen is as follows:

```
1         5         10        15        20        25
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
```

in which X represents an amino acid that has not been identified.

A comparison of the 26-residue L45 amino-terminal sequence against the PIR database (PIR Release 180, September, 1988; GenBank Release 57.0, September, 1988; NEW, November 30, 1988; DIF, November 30, 1988; SWISS PROT, November 30, 1988; and LOSPRO, November 30, 1988) did not reveal significant sequence homology with any other known sequence.

The antigen recognized by the L45 antibody is a protein antigen of about 100,000 daltons molecular weight (Mr).

Immunological Characterization

A) Western Blot Analysis

Immunoaffinity-purified L45 antigen was subjected to SDS-PAGE (7.5% acrylamide, Mini-Protean II Electrophoresis Cell, Bio-Rad, Richmond, CA), as described by Laemmli, U.K., Nature 227:680-685 (1970), and electrophoretically transferred (Mini Trans-Blot Electric Transfer Cell, Bio-Rad, Richmond, CA) to Hybond-N nylon membrane (Amersham Corp., Arlington Heights, IL), as described by Towbin, et al., Proc. Nat'l Acad. Sci. U.S.A. 76:4350-4354 (1979), except that methanol was omitted from the transfer buffer. L45 antigen was immunodetected using peroxidase-conjugated goat anti-mouse IgG as a second antibody (HyClone Lab., Logan, UT) and 4-chloro-1-napthol as chromogen (Hawkes, Anal. Biochem. 123:143-146 (1982)). Immunodetection revealed that the major band at Mr = 100,000 was specifically stained with L45 antibody.

B) Radioimmunoprecipitation

A549 cells were biosynthetically labeled with [^{3}H]-glucosamine and [^{3}H]-leucine, respectively, by incubation in RPMI 1640 media (glucose-free or leucine-free, RPMI 1640 Select-Amine Kit, GIBCO), supplemented with 10% dialyzed fetal bovine serum (FBS) for 5 hr at 37°C and chased overnight in DMEM media, supplemented with 10% FBS. The cell pellets were extracted with 50 mM sodium phosphate buffer, pH 7.2, 150 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, PMSF (10 µg/ml), aprotinin (10 µg/ml). L45 antigen was immunoprecipitated by incubating the lysates with L45 antibody for 1 hr at 4°C. The antigen antibody complex was precipitated with goat anti-mouse IgG and Pansorbin (Staphylococcus aureus cells, Calbiochem, San Diego, CA) by sequentially incubating for 30 min, each, at 4°C. The immunoprecipitate was washed 4 times with 50 mM sodium phosphate buffer, pH 7.2, 150 mM NaCl, 2 mM EDTA, 0.1% NP40, and analyzed by SDS-PAGE under reducing and non-reducing conditions and visualized by fluorography after impregnating the gel with EN^{3}HANCETM (New England Nuclear, Boston, MA).

L45 antibody specifically precipitated L45 antigen with a Mr = 100,000 in both [^{3}H]-leucine and [^{3}H]-glucosamine-labeled cells. These data demonstrate that the antigenic determinant recognized by L45 monoclonal antibody is located on a unique single-chain glycoprotein with Mr = 100,000. The L45 antigen is associated with a variety of tumor cells including lung, breast, color and ovary carcimona cells and melanoma and sarcoma cells.

It is apparent that many modifications and variations of this invention as set forth above may be made without departing from the spirit and scope. The specific embodiments described are given by way of example only and the invention is limited only by the terms of the appended claims.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. A monoclonal antibody produced by hybridoma cell line ATCC No. HB 9804, which antibody binds to a determinant site on a cell surface glycoprotein antigen of human tumor cells and antibodies which bind to the same antigenic determinant as does the monoclonal antibody produced by hybridoma cell line ATCC No. HB 9804 and compete with the monoclonal antibody produced by ATCC No. HB 9804 for binding at that antigenic determinant, Fab, $F(ab')_2$, and Fv fragments and conjugates of said antibody.

2. The monoclonal antibody of claim 1 wherein said tumor cells are carcinoma cells, melanoma cells, or sarcoma cells.

3. The monoclonal antibody of claim 2 wherein said carcinoma cells are selected from the group consisting of lung, ovary, colon and breast carcinoma cells.

4. The monoclonal antibody of claim 1 conjugated to a label capable of producing a detectable signal.

5. The monoclonal antibody of claim 4 wherein the label is selected from the group consisting of a radionuclide, an enzyme, a fluorescent agent and a chromophore.

6. A monoclonal antibody reactive with a determinant site on a cell surface glycoprotein antigen associated with human tumor cells, said antigen characterized by a molecular weight of about 100,000 daltons as determined by polyacrylamide gel electrophoresis and having an amino terminal amino acid sequence as follows:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
in which X represents an unidentified amino acid and antibodies which bind to the same antigenic determinant as does the monoclonal antibody produced by hybridoma cell line ATCC No. HB 9804 and compete with the monoclonal antibody produced by hybridoma cell line ATCC No. HB 9804 for binding at that antigenic determinant, Fab, $F(ab')_2$, and Fv fragments and conjugates of said antibody.

7. The monoclonal antibody of claim 6 produced by a hybridoma cell line.

8. The monoclonal antibody of claim 7 which is of class IgG.

9. The monoclonal antibody of claim 7 which is of subclass IgG2a.

10. The monoclonal antibody of claim 7 which is a murine antibody.

11. The monoclonal antibody of claim 6 consisting of the monoclonal antibody produced by hybridoma cell line ATCC No. HB 9804.

12. The monoclonal antibody of claim 6 which is a human antibody, or a mouse-human antibody.

13. A continuous cell line which produces a monoclonal antibody of one of claims 1 to 12, which cell line comprises: a hybridoma of a lymphocyte capable of producing antibody against said antigen and a myeloma cell.

14. The continuous cell line of claim 13, being hybridoma cell line ATCC number HB 9804.

15. The hybridoma cell line of claim 14 formed by fusing an NSI mouse myeloma cell with a mouse splenocyte obtained from a BALB/c mouse immunized with lung adenocarcinoma CH3 cells.

16. A continuous cell line which produces a monoclonal antibody of one of claims 1 to 12, wherein said monoclonal antibody binds to the same antigenic determinant as monoclonal antibody L45 produced by hybridoma cell line ATCC No. HB 9804, said cell line produced by the process of fusing a lymphocyte derived from a mouse immunized with carcinoma cells or an immunogenic determinant thereof and a mouse myeloma cell; or the process of fusing a lymphocyte derived from a human with carcinoma and a myeloma cell.

17. An immunoassay for the detection of human tumors comprising:

a) combining a monoclonal antibody reactive with a cell surface glycoprotein antigen associated with human tumor cells, said antigen characterized by a molecular weight of about 100,000 daltons as determined by polyacrylamide gel electrophoresis and having an amino terminal amino acid sequence as follows:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
in which X represents an unidentified amino acid, with a sample of tumor cells said antibody labeled so as to be capable of detection; and

b) assaying for said labeled monoclonal antibody binding to tumor cells associated with said antigen.

18. The immunoassay of claim 17 wherein said monoclonal antibody is the antibody produced by hybridoma cell line ATCC No. HB 9804.

19. The immunoassay of claim 17 wherein said antibody is labelled with a label selected from the group consisting of a radionuclide, an enzyme, a fluorescent agent and a chromophore.

20. An in vitro method for detecting tumors which comprises: contacting the monoclonal antibody of one of claims 1 to 12 with a human tissue or fluid sample, and detecting interaction of said antibody with any antigenically corresponding tumor cells or antigenic determinants thereof in said sample.

21. The method of claim 20 wherein said tumor cells are:

a) lung carcinoma cells and the human tissue is lung tissue;
b) breast carcinoma cells and the human tissue is breast tissue;
c) colon carcinoma cells and the human tissue is colon tissue;
d) ovary carcinoma cells and the human tissue is ovary tissue;
e) melanoma cells; or
f) sarcoma cells.

22. The method according to claim 20, wherein the interaction of said monoclonal antibody with said tumor cells is detected by immunohistological staining.

23. A glycoprotein antigen in substantially purified form, said antigen derived from human tumor cells and having a molecular weight of about 100,000 daltons as determined by polyacrylamide gel electrophoresis and having an amino terminal amino acid sequence as follows:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
in which X represents an unidentified amino acid and immunocomplexes of this antigen.

24. A vaccine for use in immunization against tumors comprising a recombinant virus including the DNA coding for the antigen of claim 23.

25. The vaccine of claim 24 wherein said virus is vaccinia virus.

26. A pharmaceutical composition comprising a therapeutically effective amount of an antibody of one of the claims 1 to 12 in association with a pharmaceutically acceptable vehicle.

27. The use of at least one antibody as defined in claims 1 to 12 for preparing a pharmaceutical composition for the treatment of tumors.

28. The use of claim 27, wherein said antibody is conjugated to a cytotoxic agent, a toxin or a radiopharmaceutical, or wherein said antibody is an anti-idiotype antibody.

29. The use of at least one antibody as defined in claims 1 to 12 for preparing a composition for localizing tumors in vivo.

Claims for the following Contracting State : ES

1. A method of preparing a monoclonal antibody, which method comprises providing a monoclonal antibody produced by hybridoma cell line ATCC No. HB 9804, which antibody binds to a determinant site on a cell surface glycoprotein antigen of human tumor cells and antibodies which bind to the same antigenic determinant as does the monoclonal

antibody produced by hybridoma cell line ATCC No. HB 9804 and compete with the monoclonal antibody produced by ATCC No. HB 9804 for binding at that antigenic determinant, Fab, $F(ab')_2$, and Fv fragments and conjugates of said antibody.

2. The method of claim 1 wherein said tumor cells are carcinoma cells, melanoma cells, or sarcoma cells.

3. The method of claim 2 wherein said carcinoma cells are selected from the group consisting of lung, ovary, colon and breast carcinoma cells.

4. The method of claim 1 wherein said monoclonal antibody is conjugated to a label capable of producing a detectable signal.

5. The method of claim 4 wherein the label is selected from the group consisting of a radionuclide, an enzyme, a fluorescent agent and a chromophore.

6. A method of preparing a monoclonal antibody, which method comprises providing a monoclonal antibody reactive with a determinant site on a cell surface glycoprotein antigen associated with human tumor cells, said antigen characterized by a molecular weight of about 100,000 daltons as determined by polyacrylamide gel electrophoresis and having an amino terminal amino acid sequence as follows:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
in which X represents an unidentified amino acid and antibodies which bind to the same antigenic determinant as does the monoclonal antibody produced by hybridoma cell line ATCC No. HB 9804 and compete with the monoclonal antibody produced by hybridoma cell line ATCC No. HB 9804 for binding at that antigenic determinant, Fab, $F(ab')_2$, and Fv fragments and conjugates of said antibody.

7. The method of claim 6 wherein the monoclonal antibody is produced by a hybridoma cell line.

8. The method of claim 7 wherein the monoclonal antibody is of class IgG.

9. The method of claim 7 wherein the monoclonal antibody is of subclass IgG2a.

10. The method of claim 7 wherein the monoclonal antibody is a murine antibody.

11. The method of claim 6 wherein the monoclonal antibody is produced by hybridoma cell line ATCC No. HB 9804.

12. The method of claim 6 wherein the monoclonal antibody is a human antibody, or a mouse-human antibody.

13. A method of preparing a continuous cell line which produces a monoclonal antibody of one of claims 1 to 12, which method comprises providing a cell line encompassing a hybridoma of a lymphocyte capable of producing antibody against said antigen and a myeloma cell.

14. The method of claim 13 wherein the continuous cell line is hybridoma cell line ATCC number HB 9804.

15. The method of claim 14, wherein the hybridoma cell line is formed by fusing an NSI mouse myeloma cell with a mouse splenocyte obtained from a BALB/c mouse immunized with lung adenocarcinoma CH3 cells.

16. A method of preparing a continuous cell line which produces a monoclonal antibody of one of claims 1 to 12, wherein said monoclonal antibody binds to the same antigenic determinant as monoclonal antibody L45 produced by hybridoma cell line ATCC No. HB 9804, said cell line produced by fusing a lymphocyte derived from a mouse immunized with carcinoma cells or an immunogenic determinant thereof and a mouse myeloma cell; or by fusing a lymphocyte derived from a human with carcinoma and a myeloma cell.

17. An immunoassay for the detection of human tumors comprising:

a) combining a monoclonal antibody reactive with a cell surface glycoprotein antigen associated with human tumor cells, said antigen characterized by a molecular weight of about 100,000 daltons as determined by polyacrylamide gel electrophoresis and having an amino terminal amino acid sequence as follows:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F

in which X represents an unidentified amino acid, with a sample of tumor cells said antibody labeled so as to be capable of detection; and

b) assaying for said labeled monoclonal antibody binding to tumor cells associated with said antigen.

18. The immunoassay of claim 17 wherein said monoclonal antibody is the antibody produced by hybridoma cell line ATCC No. HB 9804.

19. The immunoassay of claim 17 wherein said antibody is labeled with a label selected from the group consisting of a radionuclide, an enzyme, a fluorescent agent and a chromophore.

20. An in vitro method for detecting tumors which comprises: contacting the monoclonal antibody of one of claims 1 to 12 with a human tissue or fluid sample, and detecting interaction of said antibody with any antigenically corresponding tumor cells or antigenic determinants thereof in said sample.

21. The method of claim 20 wherein said tumor cells are:

a) lung carcinoma cells and the human tissue is lung tissue;
b) breast carcinoma cells and the human tissue is breast tissue;
c) colon carcinoma cells and the human tissue is colon tissue;
d) ovary carcinoma cells and the human tissue is ovary tissue;
e) melanoma cells; or
f) sarcoma cells.

22. The method according to claim 20, wherein the interaction of said monoclonal antibody with said tumor cells is detected by immunohistological staining.

23. A method of preparing a glycoprotein antigen in substantially purified form, which method comprises providing an antigen derived from human tumor cells and having a molecular weight of about 100,000 daltons as determined by polyacrylamide gel electrophoresis and having an amino terminal amino acid sequence as follows:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
in which X represents an unidentified amino acid and immunocomplexes of this antigen.

24. A method of preparing a vaccine for use in immunization against tumors which method comprises providing a recombinant virus including the DNA coding for the antigen of claim 23.

25. The method of claim 24 wherein said virus is vaccinia virus.

26. A method of preparing a pharmaceutical composition which method comprises providing a therapeutically effective amount of an antibody of one of the claims 1 to 12 in association with a pharmaceutically acceptable vehicle.

27. The use of at least one antibody as defined in claims 1 to 12 for preparing a pharmaceutical composition for the treatment of tumors.

28. The use of claim 27, wherein said antibody is conjugated to a cytotoxic agent, a toxin or a radiopharmaceutical, or wherein said antibody is an anti-idiotype antibody.

29. The use of at least one antibody as defined in claims 1 to 12 for preparing a composition for localizing tumors in vivo.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Monoklonaler Antikörper, produziert von der Hybridomzellinie mit der ATCC-Nr. HB 9804, wobei der Antikörper an eine Determinante auf einem Zelloberflächenglycoprotein-Antigen menschlicher Tumorzellen bindet, und Antikörper, die an dieselbe antigene Determinante binden wie der monoklonale Antikörper, der von der Hybridomzellinie mit der ATCC-Nr. HB 9804 produziert wird, und die mit dem von der Hybridomzellinie mit der ATCC-Nr. HB 9804

produzierten monoklonalen Antikörper um die Bindung an diese antigene Determinante konkurrieren, sowie Fab-, $F(ab')_2$- und Fv-Fragmente und Konjugate dieses Antikörpers.

2. Monoklonaler Antikörper nach Anspruch 1, wobei die Tumorzellen Karzinomzellen, Melanomzellen oder Sarkomzellen sind.

3. Monoklonaler Antikörper nach Anspruch 2, wobei die Karzinomzellen ausgewählt sind unter Lungen-, Ovarial-, Kolon- und Brustkarzinomzellen.

4. Monoklonaler Antikörper nach Anspruch 1, konjugiert an eine Markierung, die in der Lage ist, ein detektierbares Signal zu erzeugen.

5. Monoklonaler Antikörper nach Anspruch 4, wobei die Markierung ausgewählt ist unter einem Radionuklid, einem Enzym, einem fluoreszierenden Agens und einem Chromophor.

6. Monoklonaler Antikörper, reaktiv mit einer Determinante auf einem Zelloberflächenglycoprotein-Antigen, das mit menschlichen Tumorzellen assoziiert ist, wobei das Antigen durch ein Molekulargewicht von etwa 100 000 Dalton, bestimmt durch Polyacrylamidgelelektrophorese, charakterisiert ist und folgende aminoterminale Aminosäuresequenz aufweist:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F,
worin X für eine nicht-identifizierte Aminosäure steht, und Antikörper, die an dieselbe antigene Determinante binden wie der monoklonale Antikörper, der von der Hybridomzellinie mit der ATCC-Nr. HB 9804 produziert wird, und die mit dem von der Hybridomzellinie mit der ATCC-Nr. HB 9804 produzierten monoklonalen Antikörper um die Bindung an diese antigene Determinante konkurrieren, sowie die Fab-, $F(ab')_2$- und Fv-Fragmente und Konjugate dieses Antikörpers.

7. Monoklonaler Antikörper nach Anspruch 6, produziert von einer Hybridomzellinie.

8. Monoklonaler Antikörper nach Anspruch 7, der der Klasse IgG angehört.

9. Monoklonaler Antikörper nach Anspruch 7, der der Unterklasse IgG2a angehört.

10. Monoklonaler Antikörper nach Anspruch 7, bei dem es sich um einen murinen Antikörper handelt.

11. Monoklonaler Antikörper nach Anspruch 6, bei dem es sich um den monoklonalen Antikörper handelt, der von der Hybridomzellinie mit der ATCC-Nr. HB 9804 produziert wird.

12. Monoklonaler Antikörper nach Anspruch 6, bei dem es sich um einen menschlichen Antikörper oder um einen Maus-Mensch-Antikörper handelt.

13. Kontinuierliche Zellinie, die einen monoklonalen Antikörper nach einem der Ansprüche 1 bis 12 produziert, wobei die Zellinie umfaßt: ein Hybridom eines Lymphocyten, der in der Lage ist, Antikörper gegen das Antigen zu produzieren, und eine Myelomzelle.

14. Kontinuierliche Zellinie nach Anspruch 13, bei der es sich um die Hybridomzellinie mit der ATCC-Nummer HB 9804 handelt.

15. Hybridomzellinie nach Anspruch 14, die durch Fusionieren einer NSI-Mausmyelomzelle mit einer Mausmilzzelle, die aus einer mit Lungen-Adenokarzinom CH3-Zellen immunisierten BALB/c-Maus stammt, gebildet wird.

16. Kontinuierliche Zellinie, die einen monoklonalen Antikörper nach einem der Ansprüche 1 bis 12 produziert, wobei der monoklonale Antikörper an dieselbe antigene Determinante bindet, wie der von der Hybridomzellinie ATCC Nr. HB 9804 produzierte monoklonale Antikörper L45, und wobei die Zellinie hergestellt wird, indem man einen Lymphocyten aus einer mit Karzinomzellen oder einer immunogenen Determinante davon immunisierten Maus mit einer Mausmyelomzelle fusioniert; oder indem man einen Lymphocyten aus einem Menschen mit einem Karzinom und eine Myelomzelle fusioniert.

17. Immunoassay zur Detektion von menschlichen Tumoren, wobei man:

a) einen monoklonalen Antikörper, der gegenüber einem mit menschlichen Tumorzellen assoziierten Zelloberflächenglycoprotein-Antigen reaktiv ist, wobei das Antigen durch ein Molekulargewicht von etwa 100 000 Dalton, bestimmt durch Polyacrylamidgelelektrophorese, charakterisiert ist und folgende aminoterminale Aminosäuresequenz aufweist:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F,
worin X für eine nicht-identifizierte Aminosäure steht,
mit einer Tumorzellen-Probe vereinigt, wobei der Antikörper detektierbar markiert ist; und

b) einen Test auf den markierten monoklonalen Antikörper, der an Tumorzellen bindet, die mit dem Antigen assoziiert sind, durchführt.

18. Immunoassay nach Anspruch 17, wobei es sich bei dem monoklonalen Antikörper um denjenigen Antikörper handelt, der von der Hybridomzellinie mit der ATCC-NR. HB 9804 produziert wird.

19. Immunoassay nach Anspruch 17, wobei der Antikörper eine Markierung aufweist, die ausgewählt ist unter einem Radionuklid, einem Enzym, einem fluoreszierenden Mittel und einem Chromophor.

20. In vitro-Verfahren zum Detektieren von Tumoren, wobei man: einen monoklonalen Antikörper nach einem der Ansprüche 1 bis 12 mit einer menschlichem Gewebe- oder Flüssigkeitsprobe in Kontakt bringt und die Interaktion des Antikörpers mit antigenisch entsprechenden Tumorzellen oder antigenen Determinanten davon in der Probe detektiert.

21. Verfahren nach Anspruch 20, wobei es sich bei den Tumorzellen um:

a) Lungenkarzinomzellen und bei dem menschlichen Gewebe um Lungengewebe handelt;

b) Brustkarzinomzellen und bei dem menschlichen Gewebe um Brustgewebe handelt;

c) Kolonkarzinomzellen und bei dem menschlichen Gewebe um Kolongewebe handelt;

d) Ovarialkarzinomzellen und bei dem menschlichen Gewebe um Ovarialgewebe handelt;

e) Melanomzellen; oder

f) Sarkomzellen handelt.

22. Verfahren nach Anspruch 20, wobei die Interaktion des monoklonalen Antikörpers mit den Tumorzellen durch immunohistologische Färbung detektiert wird.

23. Glycoprotein-Antigen in im wesentlichen reiner Form, wobei das Antigen aus menschlichen Tumorzellen erhältlich ist und ein Molekulargewicht von etwa 100 000 Dalton, bestimmt durch Polyacrylamidgelelektrophorese, besitzt und folgende aminoterminale Aminosäuresequenz aufweist:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F,
worin X für eine nicht-identifizierte Aminosäure steht,
und Immunkomplexe dieses Antigens.

24. Vakzin zur Verwendung bei der Immunisierung gegen Tumoren, umfassend ein rekombinantes Virus, einschließlich der für das Antigen gemäß Anspruch 23 kodierenden DNA.

25. Vakzin nach Anspruch 24, wobei das Virus Vaccinia Virus ist.

26. Pharmazeutische Zusammensetzung, umfassend eine therapeutisch wirksame Menge eines Antikörpers nach einem der Ansprüche 1 bis 12, assoziiert mit einem pharmazeutisch akzeptablen Träger.

27. Verwendung wenigstens eines Antikörpers nach einem der Ansprüche 1 bis 12 zur Herstellung einer pharmazeutischen Zusammensetzung zur Behandlung von Tumoren.

28. Verwendung nach Anspruch 27, wobei der Antikörper mit einem cytotoxischen Agens, einem Toxin oder einem Radiopharmazeutikum konjugiert ist oder wobei der Antikörper ein Anti-Idiotyp-Antikörper ist.

29. Verwendung wenigstens eines Antikörpers nach einem der Ansprüche 1 bis 12 zur Herstellung einer Zusammensetzung zur Lokalisierung von Tumoren in vivo.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung eines monoklonalen Antikörpers, umfassend die Bereitstellung eines monoklonalen Antikörpers, produziert von der Hybridomzellinie mit der ATCC-Nr. HB 9804, wobei der Antikörper an eine Determinante auf einem Zelloberflächenglycoprotein-Antigen menschlicher Tumorzellen bindet, und von Antikörpern, die an dieselbe antigene Determinante binden wie der monoklonale Antikörper, der von der Hybridomzellinie mit der ATCC-Nr. HB 9804 produziert wird, und die mit dem von der Hybridomzellinie mit der ATCC-Nr. HB 9804 produzierten monoklonalen Antikörper um die Bindung an diese antigene Determinante konkurrieren, sowie der Fab-, $F(ab')_2$- und Fv-Fragmente und Konjugate dieses Antikörpers.

2. Verfahren nach Anspruch 1, wobei die Tumorzellen Karzinomzellen, Melanomzellen oder Sarkomzellen sind.

3. Verfahren nach Anspruch 2, wobei die Karzinomzellen ausgewählt sind unter Lungen-, Ovarial-, Kolon- und Brustkarzinomzellen.

4. Verfahren nach Anspruch 1, wobei der monoklonale Antikörper an eine Markierung konjugiert ist, die in der Lage ist, ein detektierbares Signal zu erzeugen.

5. Verfahren nach Anspruch 4, wobei die Markierung ausgewählt ist unter einem Radionuklid, einem Enzym, einem fluoreszierenden Agens und einem Chromophor.

6. Verfahren zur Herstellung eines monoklonalen Antikörpers, wobei man einen monoklonalen Antikörper bereitstellt, der mit einer Determinante auf einem Zelloberflächenglycoprotein-Antigen, das mit menschlichen Tumorzellen assoziiert ist, reagieren kann, wobei das Antigen durch ein Molekulargewicht von etwa 100 000 Dalton, bestimmt durch Polyacrylamidgelelektrophorese, charakterisiert ist und folgende aminoterminale Aminosäuresequenz aufweist:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F,
worin X für eine nicht-identifizierte Aminosäure steht, und von Antikörpern, die an dieselbe antigene Determinante binden wie der monoklonale Antikörper, der von der Hybridomzellinie mit der ATCC-Nr. HB 9804 produziert wird, und die mit dem von der Hybridomzellinie mit der ATCC-Nr. HB 9804 produzierten monoklonalen Antikörper um die Bindung an diese antigene Determinante konkurrieren, sowie der Fab-, $F(ab')_2$- und Fv-Fragmente und Konjugate dieses Antikörpers.

7. Verfahren nach Anspruch 6, wobei der monoklonale Antikörper von einer Hybridomzellinie produziert wird.

8. Verfahren nach Anspruch 7, wobei der monoklonale Antikörper der Klasse IgG angehört.

9. Verfahren nach Anspruch 7, wobei der monoklonale Antikörper der Unterklasse IgG2a angehört.

10. Verfahren nach Anspruch 7, wobei es sich bei dem monoklonalen Antikörper um einen murinen Antikörper handelt.

11. Verfahren nach Anspruch 6, wobei der monoklonale Antikörper von der Hybridomzellinie mit der ATCC-Nr. HB 9804 produziert wird.

12. Verfahren nach Anspruch 6, wobei es sich bei dem monoklonalen Antikörper um einen menschlichen Antikörper oder um einen Maus-Mensch-Antikörper handelt.

13. Verfahren zur Herstellung einer kontinuierlichen Zellinie, die einen monoklonalen Antikörper nach einem der Ansprüche 1 bis 12 produziert, wobei man eine Zellinie bereitstellt, umfassend ein Hybridom aus einem Lymphocyten, der in der Lage ist, Antikörper gegen das Antigen zu produzieren, und einer Myelomzelle.

14. Verfahren nach Anspruch 13, wobei es sich bei der kontinuierlichen Zellinie um die Hybridomzellinie mit der ATCC-Nummer HB 9804 handelt.

15. Verfahren nach Anspruch 14, wobei die Hybridomzellinie durch Fusionieren einer NSI-Mausmyelomzelle mit einer Mausmilzzelle, die aus einer mit Lungen-Adenokarzinom CH3-Zellen immunisierten BALB/c-Maus stammt, gebildet wird.

16. Verfahren zur Herstellung einer kontinuierlichen Zellinie, die einen monoklonalen Antikörper nach einem der Ansprüche 1 bis 12 produziert, wobei der monoklonale Antikörper an dieselbe antigene Determinante bindet, wie der von der Hybridomzellinie ATCC Nr. HB 9804 produzierte monoklonale Antikörper L45, und wobei die Zellinie hergestellt wird, indem man einen Lymphocyten aus einer mit Karzinomzellen oder einer immunogenen Determinante davon immunisierten Maus mit einer Mausmyelomzelle fusioniert; oder indem man einen Lymphocyten aus einem Menschen mit einem Karzinom und eine Myelomzelle fusioniert.

17. Immunoassay zur Detektion von menschlichen Tumoren, wobei man:

a) einen monoklonalen Antikörper, der gegenüber einem mit menschlichen Tumorzellen assoziierten Zelloberflächenglycoprotein-Antigen reaktiv ist, wobei das Antigen durch ein Molekulargewicht von etwa 100 000 Dalton, bestimmt durch Polyacrylamidgelelektrophorese, charakterisiert ist und folgende aminoterminale Aminosäuresequenz aufweist:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F,
worin X für eine nicht-identifizierte Aminosäure steht,
mit einer Tumorzellen-Probe vereinigt, wobei der Antikörper detektierbar markiert ist; und

b) einen Test auf den markierten monoklonalen Antikörper, der an Tumorzellen bindet, die mit dem Antigen assoziiert sind, durchführt.

18. Immunoassay nach Anspruch 17, wobei es sich bei dem monoklonalen Antikörper um denjenigen Antikörper handelt, der von der Hybridomzellinie mit der ATCC-NR. HB 9804 produziert wird.

19. Immunoassay nach Anspruch 17, wobei der Antikörper eine Markierung aufweist, die ausgewählt ist unter einem Radionuklid, einem Enzym, einem fluoreszierenden Mittel und einem Chromophor.

20. In vitro-Verfahren zum Detektieren von Tumoren, wobei man: einen monoklonalen Antikörper nach einem der Ansprüche 1 bis 12 mit einer menschlichem Gewebe- oder Flüssigkeitsprobe in Kontakt bringt und die Interaktion des Antikörpers mit antigenisch entsprechenden Tumorzellen oder antigenen Determinanten davon in der Probe detektiert.

21. Verfahren nach Anspruch 20, wobei es sich bei den Tumorzellen um:

a) Lungenkarzinomzellen und bei dem menschlichen Gewebe um Lungengewebe handelt;

b) Brustkarzinomzellen und bei dem menschlichen Gewebe um Brustgewebe handelt;

c) Kolonkarzinomzellen und bei dem menschlichen Gewebe um Kolongewebe handelt;

d) Ovarialkarzinomzellen und bei dem menschlichen Gewebe um Ovarialgewebe handelt;

e) Melanomzellen; oder

f) Sarkomzellen handelt.

22. Verfahren nach Anspruch 20, wobei die Interaktion des monoklonalen Antikörpers mit den Tumorzellen durch immunohistologische Färbung detektiert wird.

23. Verfahren zur Herstellung eines Glycoprotein-Antigens in im wesentlichen reiner Form, wobei man ein Antigen, das aus menschlichen Tumorzellen erhältlich ist und ein Molekulargewicht von etwa 100 000 Dalton, bestimmt durch Polyacrylamidgelelektrophorese, besitzt und folgende aminoterminale Aminosäuresequenz aufweist:
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F,
worin X für eine nicht-identifizierte Aminosäure steht,
und Immunkomplexe dieses Antigens bereitstellt.

24. Verfahren zur Herstellung eines Vakzins zur Verwendung bei der Immunisierung gegen Tumoren, wobei man ein rekombinantes Virus, einschließlich der für das Antigen gemäß Anspruch 23 kodierenden DNA, bereitstellt.

25. Verfahren nach Anspruch 24, wobei das Virus Vaccinia Virus ist.

26. Verfahren zur Herstellung einer pharmazeutischen Zusammensetzung, wobei man eine therapeutisch wirksame Menge eines Antikörpers nach einem der Ansprüche 1 bis 12, assoziiert mit einem pharmazeutisch akzeptablen Träger, bereitstellt.

27. Verwendung wenigstens eines Antikörpers nach einem der Ansprüche 1 bis 12 zur Herstellung einer pharmazeutischen Zusammensetzung zur Behandlung von Tumoren.

28. Verwendung nach Anspruch 27, wobei der Antikörper mit einem cytotoxischen Agens, einem Toxin oder einem Radiopharmazeutikum konjugiert ist oder wobei der Antikörper ein Anti-Idiotyp-Antikörper ist.

29. Verwendung wenigstens eines Antikörpers nach einem der Ansprüche 1 bis 12 zur Herstellung einer Zusammensetzung zur Lokalisierung von Tumoren in vivo.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Anticorps monoclonal produit par la lignée de cellules d'hybridome ATCC N°HB 9804, lequel anticorps se lie à un site déterminant sur un antigène de glycoprotéine de surface de cellule des cellules de tumeur humaine et des anticorps qui se lient au même déterminant antigénique que l'anticorps monoclonal produit par la lignée des cellules d'hybridome ATCC N°HB 9804 et entrent en compétition avec l'anticorps monoclonal produit par ATCC N°HB 9804 pour une liaison à ce déterminant antigénique, les fragments Fab, $F(ab')_2$, et Fv et les conjugués dudit anticorps.

2. Anticorps monoclonal de la revendication 1 où lesdites cellules de tumeur sont des cellules de carcinome, des cellules de mélanome, ou des cellules de sarcome.

3. Anticorps monoclonal de la revendication 2 où lesdites cellules de carcinome sont sélectionnées dans le groupe consistant en cellules de carcinome de poumon, d'ovaire, du côlon et du sein.

4. Anticorps monoclonal de la revendication 1 conjugué à un marqueur capable de produire un signal détectable.

5. Anticorps monoclonal de la revendication 4 où le marqueur est sélectionné dans le groupe consistant en un radionuclide, une enzyme, un agent fluorescent et un chromophore.

6. Anticorps monoclonal réactif avec un site déterminant sur un antigène de glycoprotéine de surface de cellule associé à des cellules de tumeur humaine, ledit antigène étant caractérisé par un poids moléculaire d'environ 100000 daltons en déterminant par électrophorèse sur gel de polyacrylamide et ayant une séquence d'acides aminés amino terminale comme suit :
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
où X représente un acide aminé non identifié et des anticorps qui se lient au même déterminant antigénique que le fait l'anticorps monoclonal produit par la lignée de cellules d'hybridome ATCC N°HB 9804 et entrent en compétition avec l'anticorps monoclonal produit par la lignée de cellules d'hybridome ATCC N°HB 9804 pour une liaison à ce déterminant antigénique, les fragments Fab, $F(ab')_2$, et Fv et les conjugués dudit anticorps.

7. Anticorps monoclonal de la revendication 6 produit par une lignée de cellules d'hybridome.

8. Anticorps monoclonal de la revendication 7 qui est de la classe d'IgG.

9. Anticorps monoclonal de la revendication 7 qui est de la sous-classe IgG2a.

10. Anticorps monoclonal de la revendication 7 qui est l'anticorps de murin.

11. Anticorps monoclonal de la revendication 6 consistant en l'anticorps monoclonal produit par la lignée de cellules d'hybridome ATCC N°HB 9804.

12. Anticorps monoclonal de la revendication 6 qui est un anticorps humain, ou un anticorps souris-humain.

13. Lignée de cellules continue qui produit l'anticorps monoclonal de l'une des revendications 1 à 12, laquelle lignée de cellules comprend : un hybridome d'un lymphocyte capable de produire un anticorps contre ledit antigène et une cellule de myélome.

14. Lignée de cellules continue de la revendication 13, qui une lignée de cellules d'hybridome ATCC numéro HB 9804.

15. Lignée de cellules d'hybridome de la revendication 14 formée par fusion d'une cellule de myélome de souris NSI avec un splénocyte de souris obtenu d'une souris BALB/c immunisée avec les cellules CH3 d'adénocarcinome du poumon.

16. Lignée de cellules continue qui produit un anticorps monoclonal de l'une des revendications 1 à 12, où ledit anticorps monoclonal se lie au même déterminant antigénique que l'anticorps monoclonal L45 produit par la lignée de cellules d'hybridome ATCC N°HB 9804, ladite lignée de cellules produite par le procédé de fusion d'un lymphocyte dérivé d'une souris immunisée avec des cellules de carcinome ou un déterminant immunogène et une cellule de myélome de souris; ou bien le procédé de fusion d'un lymphocyte dérivé d'un humain avec un carcinome et une cellule de myélome.

17. Immunodosage pour la détection de tumeurs humaines consistant à :

a) combiner un anticorps monoclonal réactif avec un antigène de glycoprotéine de surface de cellule associé à des cellules de tumeur humaine, ledit antigène étant caractérisé par un poids moléculaire d'environ 100 000 daltons déterminé par électrophorèse sur gel de polyacrylamide et ayant une séquence d'acides aminés amino terminale comme suit :
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
où X représente un acide aminé non identifié, avec un échantillon de cellules de tumeur, ledit anticorps étant marqué afin d'être capable de détection; et

b) doser ledit anticorps monoclonal marqué se liant aux cellules de tumeur en association avec ledit antigène.

18. Immunodosage de la revendication 17 où ledit anticorps monoclonal est l'anticorps produit par la lignée de cellules d'hybridome ATCC N°HB 9804.

19. Immunodosage de la revendication 17 où ledit anticorps est marqué par un marqueur sélectionné dans le groupe consistant en un radionuclide, une enzyme, un agent fluorescent et un chromophore.

20. Méthode in vitro pour la détection de tumeurs qui comprend :
la mise en contact de l'anticorps monoclonal de l'une des revendications 1 à 12 avec un échantillon de tissu ou de fluide humain, et la détection de l'interaction dudit anticorps avec toutes les cellules de tumeur antigéniquement correspondantes ou les déterminants antigéniques dans ledit échantillon.

21. Méthode de la revendication 20 où lesdites cellules de tumeur sont :

a) des cellules de carcinome de poumon et le tissu humain et le tissu du poumon;

b) des cellules de carcinome du sein et le tissu humain est le tissu du sein;

c) des cellules de carcinome de côlon et le tissu humain et le tissu du côlon;

d) des cellules de carcinome d'ovaire et le tissu humain est du tissu d'ovaire;

e) des cellules de mélanome; ou

f) des cellules de sarcome.

22. Méthode selon la revendication 20, où l'interaction dudit anticorps monoclonal avec lesdites cellules de tumeur est détectée par une coloration immunohistologique.

23. Antigène de glycoprotéine sous une forme sensiblement purifiée, ledit antigène étant dérivé de cellules de tumeur humaine et ayant un poids moléculaire d'environ 100000 daltons en déterminant par électrophorèse sur gel de polyacrylamide et ayant une séquence d'acides aminés amino terminale comme suit :
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
où X représente un acide aminé non identifié et les immunocomplexes de cet antigène.

24. Vaccin à utiliser pour une immunisation contre des tumeurs comprenant un virus recombinant comprenant l'ADN codant pour l'antigène de la revendication 23.

25. Vaccin de la revendication 24 où ledit virus est le virus de la vaccine.

26. Composition pharmaceutique comprenant une quantité thérapeutiquement efficace d'un anticorps de l'une des revendications 1 à 12 en association avec un véhicule pharmaceutiquement acceptable.

27. Utilisation d'au moins un anticorps tel que défini aux revendications 1 à 12 pour la préparation d'une composition pharmaceutique pour le traitement des tumeurs.

28. Utilisation de la revendication 27 où ledit anticorps est conjugué à un agent cytotoxique, une toxine ou un produit radiopharmaceutique, ou bien où ledit anticorps est un anticorps anti-idiotype.

29. Utilisation d'au moins un anticorps tel que défini aux revendications 1 à 12 pour la préparation d'une composition pour la localisation de tumeurs in vivo.

Revendications pour l'Etat contractant suivant : ES

1. Méthode de préparation d'un anticorps monoclonal, laquelle méthode consiste à prévoir un anticorps monoclonal produit par la lignée de cellule d'hybridome ATCC N° HB 9804, lequel anticorps se lie à un site déterminant sur un antigène de glycoprotéine de surface de cellule des cellules de tumeur humaine et des anticorps qui se lient au même déterminant antigénique que l'anticorps monoclonal produit par la lignée des cellules d'hybridome ATCC N°HB 9804 et entrent en compétition avec l'anticorps monoclonal produit par ATCC N°HB 9804 pour une liaison à ce déterminant antigénique, les fragments Fab, $F(ab')_2$, et Fv et les conjugués dudit anticorps.

2. Méthode de la revendication 1 où lesdites cellules de tumeur sont des cellules de carcinome, des cellules de mélanome, ou des cellules de sarcome.

3. Méthode de la revendication 2 où lesdites cellules de carcinome sont sélectionnées dans le groupe consistant en cellules de carcinome de poumon, d'ovaire, du côlon et du sein.

4. Méthode de la revendication 1 où ledit anticorps monoclonal est conjugué à un marqueur capable de produire un signal détectable.

5. Méthode de la revendication 4 où le marqueur est choisi dans le groupe consistant en un radionuclide, une enzyme, un agent fluorescent et un chromophore.

6. Méthode de préparation d'un anticorps monoclonal, laquelle méthode consiste à prévoir un anticorps monoclonal réactif avec un site déterminant sur un antigène de glycoprotéine de surface de cellule associé à des cellules de tumeur humaine, ledit antigène étant caractérisé par un poids moléculaire d'environ 100 000 daltons en déterminant par électrophorèse sur gel de polyacrylamide et ayant une séquence d'acides aminés amino terminale comme suit :
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
où X représente un acide aminé non identifié et des anticorps qui se lient au même déterminant antigénique que le fait l'anticorps monoclonal produit par la lignée de cellules d'hybridome ATCC N°HB 9804 et entrent en compétition avec l'anticorps monoclonal produit par la lignée de cellules d'hybridome ATCC N°HB 9804 pour une liaison à ce déterminant antigénique, les fragments Fab, $F(ab')_2$, et Fv et les conjugués dudit anticorps.

7. Méthode de la revendication 6 où l'anticorps monoclonal est produit par une lignée de cellules d'hybridome.

8. Méthode de la revendication 7 où l'anticorps monoclonal est de la classe d'IgG.

9. Méthode de la revendication 7 où l'anticorps monoclonal est de la sous-classe IgG2a.

10. Méthode de la revendication 7 où l'anticorps monoclonal est un anticorps de murin.

11. Méthode de la revendication 6 où l'anticorps monoclonal est produit par la lignée de cellules d'hybridome ATCC N°HB 9804.

12. Méthode de la revendication 6 où l'anticorps monoclonal est un anticorps humain ou un anticorps souris-humain.

13. Méthode de préparation d'une lignée de cellules continue qui produit un anticorps monoclonal de l'une des revendications 1 à 12, laquelle méthode consiste à prévoir une lignée de cellules contenant un hybridome d'un lymphocyte capable de produire un anticorps contre ledit antigène et une cellule de myélome.

14. Méthode de la revendication 13 où la lignée de cellules continue est la lignée de cellules d'hybridome ATCC numéro HB 9804.

15. Méthode de la revendication 14, où la lignée de cellules d'hybridome est formée par fusion d'une cellule de myélome de souris NSI avec un splénocyte de souris obtenu d'une souris BALB/c immunisée avec des cellules CH3 d'adénocarcinome du poumon.

16. Méthode de préparation d'une lignée de cellules continue qui produit un anticorps monoclonal de l'une des revendications 1 à 12, où ledit anticorps monoclonal se lie au même déterminant antigénique que l'anticorps monoclonal L45 produit par la lignée de cellules d'hybridome ATCC N°HB 9804, ladite lignée de cellules produite par le procédé de fusion d'un lymphocyte dérivé d'une souris immunisée avec des cellules de carcinome ou un déterminant immunogène et une cellule de myélome de souris; ou bien le procédé de fusion d'un lymphocyte dérivé d'un humain avec un carcinome et une cellule de myélome.

17. Immunodosage pour la détection de tumeurs humaines consistant à :

a) combiner un anticorps monoclonal réactif avec un antigène de glycoprotéine de surface de cellule associé à des cellules de tumeur humaine, ledit antigène étant caractérisé par un poids moléculaire d'environ 100 000 daltons déterminé par électrophorèse sur gel de polyacrylamide et ayant une séquence d'acides aminés amino terminale comme suit :
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
où X représente un acide aminé non identifié, avec un échantillon de cellules de tumeur, ledit anticorps étant marqué afin d'être capable de détection; et

b) doser ledit anticorps monoclonal marqué se liant aux cellules de tumeur en association avec ledit antigène.

18. Immunodosage de la revendication 17 où ledit anticorps monoclonal est l'anticorps produit par la lignée de cellules d'hybridome ATCC N°HB 9804.

19. Immunodosage de la revendication 17 où ledit anticorps est marqué par un marqueur sélectionné dans le groupe consistant en un radionuclide, une enzyme, un agent fluorescent et un chromophore.

20. Méthode in vitro pour la détection de tumeurs qui comprend :
la mise en contact de l'anticorps monoclonal de l'une des revendications 1 à 12 avec un échantillon de tissu ou de fluide humain et la détection de l'interaction dudit anticorps avec toutes les cellules de tumeur antigéniquement correspondantes ou les déterminants antigéniques dans ledit échantillon.

21. Méthode de la revendication 20 où lesdites cellules de tumeur sont :

a) des cellules de carcinome de poumon et le tissu humain et le tissu du poumon;

b) des cellules de carcinome du sein et le tissu humain est le tissu du sein;

c) des cellules de carcinome de côlon et le tissu humain et le tissu du côlon;

d) des cellules de carcinome d'ovaire et le tissu humain est du tissu d'ovaire;

e) des cellules de mélanome; ou

f) des cellules de sarcome.

22. Méthode selon la revendication 20, où l'interaction dudit anticorps monoclonal avec lesdites cellules de tumeur est détectée par une coloration immunohistologique.

23. Méthode de préparation d'un antigène de glycoprotéine sous une forme sensiblement purifiée, laquelle méthode consiste à prévoir un antigène dérivé de cellules de tumeur humaine et ayant un poids moléculaire d'environ 100000 daltons en déterminant par électrophorèse sur gel de polyacrylamide et ayant une séquence d'acides aminés amino terminale comme suit :
W-Y-T-V-N-S-A-Y-G-D-T-I-I-I-P-X-R-L-D-V-P-Q-N-L-M-F
où X représente un acide aminé non identifié et les immunocomplexes de cet antigène.

24. Méthode de préparation d'un vaccin utilisé pour une immunisation contre des tumeurs, laquelle méthode consiste à prévoir un virus recombinant comprenant l'ADN codant pour l'antigène de la revendication 23.

25. Méthode de la revendication 24 où ledit virus est le virus de la vaccine.

26. Méthode de préparation d'une composition pharmaceutique laquelle méthode consiste à prévoir une quantité thérapeutiquement efficace d'un anticorps de l'une des revendications 1 à 12 en association avec un véhicule pharmaceutiquement acceptable.

27. Utilisation d'au moins un anticorps tel que défini aux revendications 1 à 12 pour la préparation d'une composition pharmaceutique pour le traitement des tumeurs.

28. Utilisation de la revendication 27, où ledit anticorps est conjugué à un agent cytotoxique, une toxine ou un produit radiopharmaceutique, ou bien où ledit anticorps est un anticorps anti-idiotype.

29. Utilisation d'au moins un anticorps tel que défini dans les revendications 1 à 12 pour la préparation d'une composition pour la localisation des tumeurs in vivo.